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The significance of splenectomy in experimental swine models of controlled hemorrhagic shock

To the Editor:

Animal models of hemorrhagic shock are the cornerstone in understanding the complexities of molecular and cellular milieu associated with hemorrhage and in searching for new therapies. In the swine hemorrhage model, splenectomy is at times performed as part of the experimental proce dure as in the recent article by Kheirabadi et al.,[1] in which the authors used splenectomized swine to assess the efficacy of a Combat Ready Clamp to control hemorrhage and blood loss. Splenectomy has been advocated based on the notion that during hemorrhage, there is a release of variable amounts of sequestered erythrocytes, clotting factors, and platelets from the spleen.[2] However, the necessity of splenectomy for a robust model of hemorrhage is not unanimously established, and a large number of experimental hemorrhage studies have been conducted in nonsplenectomized animals.[3] In a letter to the editor, Devlin et al.[4] raised the question related to the necessity of splenectomy in these animal models and additionally stated that ''splenectomy may introduce a more confounding variable than what researchers are seeking to avoid with the procedure."

To delineate the role of the spleen in experimental hemorrhage models, we performed a controlled hemorrhage in a well established swine model.[5] We compared two groups of swine. In one group, a splenectomy was performed before the induction of the hemorrhage, and in the other group, animals did not receive a splenectomy (control group). In both groups, a controlled hemorrhage was performed in a 20 minute time period with blood withdrawn 1.42 mL/kg per minute during the first 7 minutes and then withdrawn 0.76 mL/kg per minute during the next 13 minutes.[5] Following induction of hemor rhage, animals were observed for a period of 120 minutes, during which a series of physi ological and biochemical parameters were recorded. In both groups, there was a rapid drop in mean arterial pressure (MAP) culmi nating in a 55% reduction in MAP by the end of the 20 minute bleeding period (end of hemorrhage [EH]) (Supplemental Digital Con tent [SDC], http://links.lww.com/TA/A295, Fig. 1A).The systolic blood pressure (SBP) dropped to 60% in the nonsplenectomy group and 51% in the splenectomy group (SDC, http://links.lww.com/TA/A295, Fig. 1B), and SvO_2 decreased more than 40% in both groups of animals. There were no changes in the heart rate (HR) from baseline (BL) in any of the two groups (SDC, http://links.lww.com/TA/A295, Fig. 1C). At the end of the 20 minute of hem orrhage, compensatory mechanisms were ob served in both groups of animals to modulate the cardiopulmonary parameters, which resulted in a peak of MAP at 49 mm Hg and 54 mm Hg, SBP of 60 mm Hg and 68 mm Hg, and HR of 149 beats per minute and 142 beats per minute in the nonsplenectomized and splenectomized groups of animals, respectively, at the end of the experiment (Fig. 1*A C*). A two way repeated measures analysis of vari ance was performed for each factor comparing splenectomy versus nonsplenectomy over time. For factors with a significant interac tion effect, pairwise comparisons were per formed with a tukey adjustment for multiple test corrections. No statistically significant differences were detected between the sple nectomized versus nonsplenectomized groups at any time point during the 20 minute period of the hemorrhage or the 120 minute posthemorrhagic shock observation period. Total hemoglobin was not affected by sple nectomy and was in the same range (9 g/dL) in both groups of swine, with no statist ically significant differences at any time point. We were unable to detect differences in serum lactate levels between the groups. These data suggest that in a nonsurvival swine model of controlled hemorrhage, spleen re moval does not significantly affect physiologic parameters during hemorrhagic shock, impor tantly hemoglobin and arterial blood pressure. These observations should be carefully eval uated in other experimental models of hem orrhage (e.g., uncontrolled hemorrhage, models with concomitant blunt or orthopedic trauma, and survival studies) and other species (e.g., canine or *Ovis aries* models).

*The authors declare no conflicts of interest.

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Re: The significance of splenectomy in experimental swine models of hemorrhagic shock

In Reply:

We appreciate the opportunity to respond to the letter to the editor by Bebarta et al. regarding our recent article. The authors raise a long standing controversy of whether splenectomy is a necessary procedure before conducting hemorrhage studies in pigs. To evaluate the necessity of splenectomy, they performed a controlled hemorrhage experi ment (20 mL/kg blood drawn in 20 minutes) in two groups of pigs in which one group was splenectomized and the other one was not. Their results showed no significant differences in hemodynamics or hemoglobin levels to hem orrhage between the two groups during the 2 hour observation period.

A review of the literature shows that these are not new findings. As early as1938, it was shown that there was no difference in animals'

ability to maintain their blood pressure when subjected to hemorrhage at 20 mL/kg or less regardless if they were splenectomized or not.[1] In contrast, in splenectomized or intact pigs or dogs that were subjected to more severe hemorrhage (≥30%), the hemodynamic compensatory mechanisms were significantly different between groups.[2,3] In addition, whether splenic contraction occurs in response to hemorrhage also depends on anesthetic regi mens,[4] but this was not specified in the authors' methods.

Other studies reported that the pig's spleen similar to that of the cat and dog but unlike that of a human sequesters up to 20% to 25%[5] of animal's blood volume and is able to autotransfuse up to 5 mL/kg to 8 mL/kg[4] blood under severe hemorrhage shock conditions. At our institute, we have seen significant differences in the spleen size among isoflurane anesthetized pigs with similar body weights (38 ± 2 kg). At the conclusion of some of our hemorrhage experiments (when no splenectomy was performed), the spleen had significantly contracted to half its normal size and was almost completely depleted of its blood content when the animal lost more than 50% of its blood volume. The variation in spleen sizes is indicative of differences in the capacity of the organ to transfuse different volumes of blood into the circulation,[2] thereby causing more variability in physiologic measurements. We prefer to perform a splenectomy in our hemorrhage/resuscitation experiments to avoid this potential variability.

We usually conduct a lethal level of hem orrhage to use survival time as an end point for determining whether a treatment (hemostatic bandage or resuscitation solution) is effective. In some of our experimental models (e.g., femoral artery injury, Grade V liver injury), the animal may lose as much as 25 mL/kg blood during the first minute of uncontrolled bleeding, which is higher than the slow controlled hemorrhage performed by Bebarta et al. We have found that once the hemorrhage volume exceeds 50%, the mortality curve be comes very steep and sensitive to every addi tional milliliter of blood lost. Thus, having the contractile spleen capable of transfusing vari able amounts of blood impairs our ability to determine which treatment is more efficacious in reducing blood loss and improving survival. The increase in variability also requires testing a larger number of animals per group to achieve sufficient power for statistical analysis. Thus, we concur with the current findings of Bebarta et al. but caution them should they investigate hemorrhages greater than 30%.

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The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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Re: The significance of splenectomy in experimental swine models of hemorrhagic shock

In Reply:

We would like to thank Bebarta et al. for their interest in our study[1] and the relevance of their findings. During the 20 minute period of hemorrhage and through out the following 120 minutes, the authors have documented no difference in mean arterial pressure, systolic blood pressure, and heart rate between the splenectomized and nonsplenectomized swine. It is thus unlikely that prehemorrhagic splenectomy would have altered our results or improved the clinical relevance of our model. We are grateful to Bebarta et al. for this interesting comment, which neither challenges nor modifies our main conclusions. Their letter also discusses a major aspect of experimen tal hemorrhagic shock models that is still debated, that is, the choice to splenectomize or not to splenectomize large animals before induction of blood withdrawal.[2]

In swine, splenic red blood cell seques tration is estimated to be in the 5 mL/kg range and could be mobilized in the circulation following stimuli that release catecholamine such as physical restraint or epinephrine injection.[3] As spleen contrac tion does not usually occur in humans, splenectomy was advocated in swine to get closer to human hemorrhagic shock. How ever, the question still arises as to whether this small amount of plasma and erythro cytes has a relevant clinical implication and justifies splenectomy in a swine model of hemorrhage. Moreover, some recent findings suggest that splenic contraction may also occur in human under some stressful circum stances.[4] Because it requires laparotomy, organ manipulation, vascular control and spleen removal, prehemorrhagic splenectomy consti tutes a surgical trauma that could enhance the inflammatory response to hemorrhage (two hit model) and induce a vasoplegic state.

From a clinical point of view, performing splenectomy before the index hemorrhage is also disputable. Indeed, in the large majority of trauma cases, surgical removal of the spleen only occurs after hemorrhage has declared. The prehemorrhagic swine splenectomy model only reproduces the rare clinical scenario where hemorrhagic shock follows an elec tive surgical splenectomy.

In our model, we chose not to splenec tomize animals submitted to hemorrhagic shock and resuscitation.[1] We believe that splenectomy would not have altered the close relationship linking the stroke volume to the pulse pressure heart rate ratio during the hemorrhagic phase. Indeed, as splenec tomy alters neither arterial compliance nor arterial elastance, the relationship between stroke volume and pulse pressure should remain constant. Furthermore, no argument suggests that splenectomy should induce different chronotropic response during sub sequent hemorrhagic shock. Even in rodents where splenectomy was shown to reduce hemodynamic tolerance to blood loss, splenectomized rats demonstrate no signifi cant difference in arterial pressure and heart rate compared with sham operated animals.[5] However, there is a hypothetical possibility that splenic contraction during norepineph rine infusion may increase mean circulatory filling pressure and pressure response in intact swine compared with splenectomized animals, as demonstrated in rats.[5]

Finally, we agree that the effect of sple nectomy on complex hemodynamic derived variables should be assessed in other experi mental models (uncontrolled hemorrhagic shock, associated soft tissue injury) and in other animal species. We wish to stress the fact that our experimental study[1] was designed to mimic the clinical setting of a bleeding patient under general anesthesia, as far as possible.

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